Review

Marine biotoxins and its detection

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The incidences of intoxication due to the consumption of marine foods have been increasing in recent years. This is due to the presence of biotoxins in foods of marine origin. The biotoxins will be accumulated in the marine foods due to the consumption of toxic biota of marine origin. When this contaminated food is taken by the humans or animals, those toxins will be transferred to them causing intoxication and lethality. Among these intoxications, most of them are caused by the harmful algal blooms (HAB). In order to avoid the harmful effects from marine biotoxins, it is necessary to have the proper knowledge. In this manuscript, the different types of biotoxins, source of intoxication, characteristics of toxins, detection and control measures are discussed in detail.

Key words: Harmful algal blooms, harmful algal blooms (HAB), ciguatara fish poisoning (CFP), paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) blooming, detection.

INTRODUCTION

Microscopic planktonic algae of the world's oceans are critical food for filter-feeding bivalve shellfish (oysters, mussels, scallops and clams) as well as for the larvae of commercially important crustaceans and fin fish. Over the last several decades, countries throughout the world have experienced an escalating trend in the incidence of "harmful algal blooms" (HABs) (Anderson, 1989; Hallegraeff, 1993). HAB events are characterized by the proliferation and occasional dominance of particular species of toxic or harmful algae. When toxic algae are filtered from the water as food by shellfish, their toxins accumulate in those shellfish to levels that can be lethal to humans or other consumers. Another type of HAB impact occurs when marine fauna are killed by algal species that release toxins and other compounds into the water. HABs include species of microscopic, usually single celled eukaryotic plants that live in estuarine and marine waters. A "bloom" occurs when algae grow very quickly or "bloom" and accumulate into dense visible patches near the surface of the water (National Office for Marine Biotoxins and Harmful Algal Blooms, 1999)

During the past two decades, the frequency, intensity and geographic distribution of harmful algal blooms has increased, along with the number of toxic compounds found in the marine food chain. Different explanations for this trend have been given such as increased scientific awareness of toxic algal species, increased utilization of coastal waters for aquaculture, transfer of shellfish stocks

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from one area to another, cultural eutrophication from domestic, industrial and agricultural wastes, increased mobility of humic substances and trace metals from soil due to deforestation and/or by acid precipitation (acid rain), and unusual climatic conditions (Hallegraeff et al., 1995).

A poorly defined but potentially significant concern relates to sublethal, chronic impacts from toxic HABs that can affect the structure and function of ecosystems. Adult fish can be killed by the millions in a single outbreak, with long- and short-term ecosystem impacts (Okaichi et al., 1989; Kim et al., 1999). Likewise, larval or juvenile stages of fish or other commercially important species can experience mortalities from algal toxins (White et al., 1989). HABs also cause mortalities of wild fish, seabirds, whales, dolphins and other marine animals. Non-toxic blooms of algae can cause harm, often due to the high biomass that some blooms achieve, and the deposition and decay of that biomass, leading to anoxia. Chronic toxin exposure may have long-term consequences that are critical with respect to the sustainability or recovery of natural populations at higher trophic levels (Ramsdell et al., 2005). Only a few of the many thousands of species of algae are associated regularly with toxic or harmful algal blooms (National Office for Marine Biotoxins and Harmful Algal Blooms, 1999). Shellfish poisoning syndromes include Ciguatara fish poisoning (CFP) and paralytic (PSP), diarrhetic (DSP), ciguatara (CFP), neurotoxic (NSP) and amnesic (ASP) shellfish poisoning based on human symptoms.

TOXINS

Ciguatara fish poisoning (CFP)

Ciguatera poisoning in humans and domestic animals is caused by potent neurotoxins produced by benthic dinoflagellates including *Gambierdiscus toxicu*, *Prorocentrum concavum*, *Prorocentrum hoffmannianum*, *Prorocentrum lima*, *Ostreopsis lenticularis*, *Ostreopsis siamensis*, *Coolia monotis*, *Thecadinium* and *Amphidinium carterae.* In the tropics and subtropics toxic dinoflagellates living on coral reefs are eaten by small herbivorous fish grazing on coral which in turn are eaten by larger carnivores. The poisons move up the food chain into the organs of larger top-order predators such as coral trout, red bass, chinaman fish, mackerels and moray eels and cause ciguatera fish poisoning, CFP, in people who eat these fish (Kim, 1999; Klöpper et al., 2003; Leikin, and Paloucek, 1998).

Toxins produced: Ciguatoxin, Maitotoxin

CFP produces gastrointestinal, neurological and cardiovascular symptoms. Generally, diarrhea, vomiting and abdominal pain occur initially, followed by neurological dysfunction including reversal of tempe-rature sensation, muscular aches, dizziness, anxiety, sweating and numbness and tingling of the mouth and digits. Paralysis and death have been documented, but symptoms are usually less severe although debilitating. Recovery time is variable, and may take weeks, months, or years. Rapid treatment (within 24 h) with mannitol reported to relieve some symptoms. There is no antidote, supportive therapy is the rule, and survivors recover. Absolute prevention of intoxication depends upon complete abstinence from eating any tropical reef fish, since there is currently no easy way to measure routinely ciguatoxin or maitotoxin in any seafood product prior to consumption, (Nielsen and Tonseth, 1991; Partensky and Sournia, 1986; Partensky et al., 1988; Partensky et al., 1991; Passow, 1991; Perez et al., 2001; Rafuse et al., 2004; Schnorf et al., 2002; Tangen, 1977; Taylor et al., 1995; Tillmann, 2004).

Chemical properties

Ciguatoxins are lipid-soluble polyether compounds consisting of 13 to 14 rings fused by ether linkages into a most rigid ladder-like structure (Figure 1). They are relatively heat-stable molecules that remain toxic after cooking and exposure to mild acidic and basic conditions. Ciguatoxins arise from biotransformation in the fish of precursor gambier toxins (Lehane and Lewis, 2000; Lehane, 2000).

In areas in the Pacific, the principal and most potent ciguatoxin is Pacific ciguatoxin-1 (P-CTX-1, mol. wt. 1112). Its likely precursor is gambiertoxin-4B (GTX-4B). The main ciguatoxins in the Pacific, P-CTX-1, P-CTX-2 and P-CTX-3, are present in fish in different relative amounts (Lehane and Lewis, 2000; Lehane, 2000). Caribbean (and Indian Ocean) ciguatoxins differ from Pacific ciguatoxins. Caribbean CTX-1 (C-CTX-1) is less polar than P-CTX-1. Structures of two Caribbean ciguatoxins (C-CTX-1 and C-CTX-2) were elucidated in 1998. The structures of more than 20 congeners of ciguatoxin were elucidated. Structural modifications were mainly seen in the both termini of the toxin molecules and mostly by oxidation (Naoki et al., 2001; Yasumoto et al., 2000). Multiple forms of ciguatoxin with minor molecular differences and pathogenicity were described. CTX-1 is the major toxin found in carnivorous fish and poses a human health risk at levels above 0.1 µg/kg fish (De Fouw et al., 1999). The energetically less favored epimers, P-CTX-2 (52-epi P-CTX-3), P-CTX-4A (52-epi P-CTX-4B) and C-CTX-2 (56-epi C-CTX-1) are indicated in parenthesis. 2, 3-Dihydroxy P-CTX-3C and 51-hydroxy P-CTX-3C have also been isolated from Pacific fish (Lewis, 2001). Various species of parrotfish have previously been reported to contain a toxin less polar than CTX-1, named scaritoxin. Judging from the reported
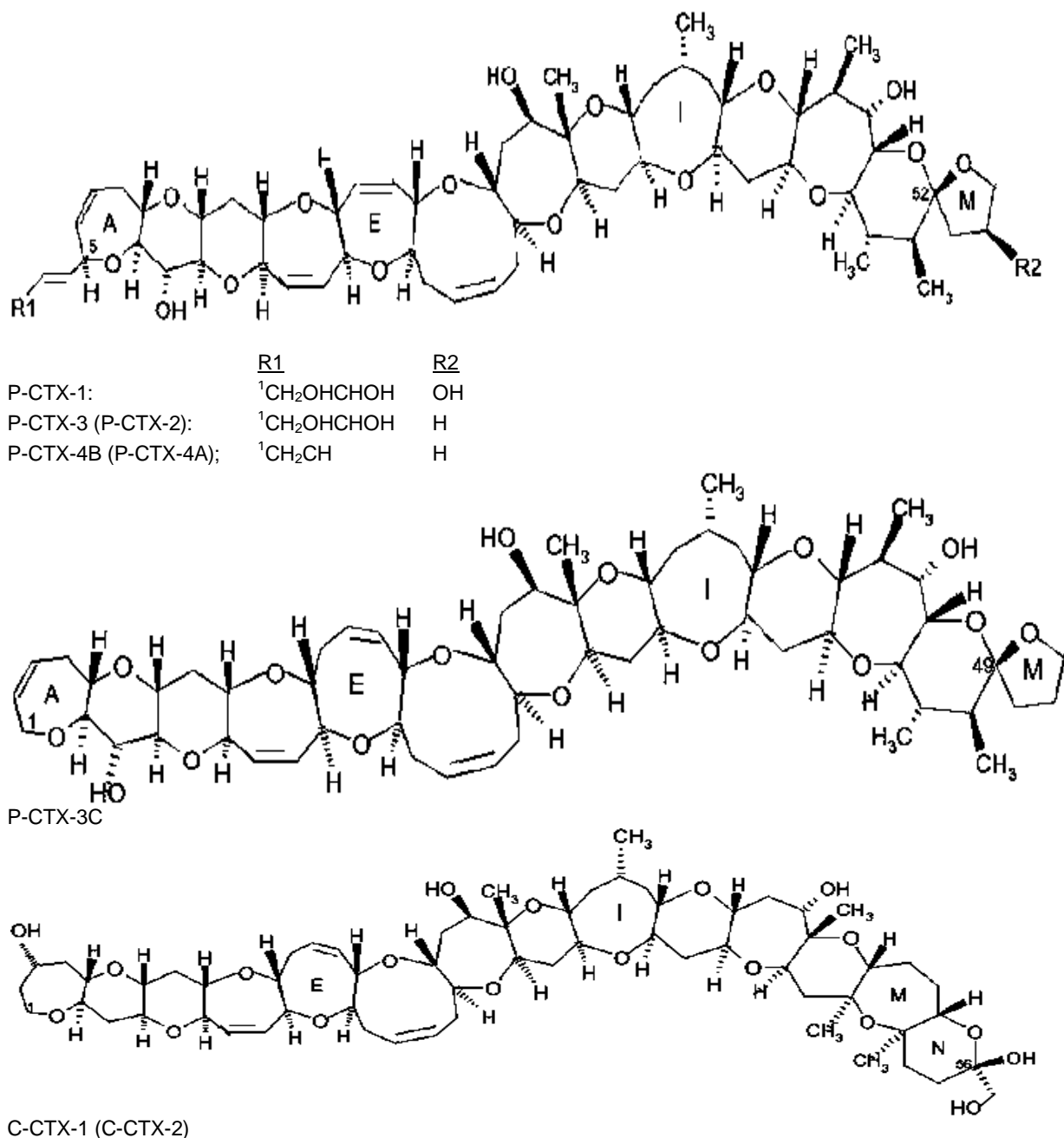
	R1	R2
P-CTX-1:	$^1CH_2OHCHOH$	OH
P-CTX-3 (P-CTX-2):	$^1CH_2OHCHOH$	H
P-CTX-4B (P-CTX-4A);	$^1CH_2CH$	H

P-CTX-3C

C-CTX-1 (C-CTX-2)

Figure 1. Structure of Pacific (P) and Caribbean (C) ciguatoxins (CTXs). Source: Yasumoto et al., 2000 and Lewis, 2001.

chromatographic properties, scaritoxin seems to correspond to a mixture of CTX-4A and CTX-4B (De Fouw et al., 1999).

Diarrhetic shellfish poisoning (DSP)

Diarrhetic shellfish poisoning (DSP) is produced by dinoflagellates in the genera *Dinophysis* and *Prorocentrum* like *Dinophysis*, *Prorocentrum*, *Dinophysis*

fortii, *Dinophysis acuminata*, *Dinophysis norvegica*, *Dinophysis acuta* (Murakami et al., 1982; Lee et al., 1989; Jackson et al., 1993).

Toxin produced: Okadaic acid

DSP produces gastrointestinal symptoms, usually beginning within 30 min to a few hours after consumption of toxic shellfish. The illness, which is not fatal, is
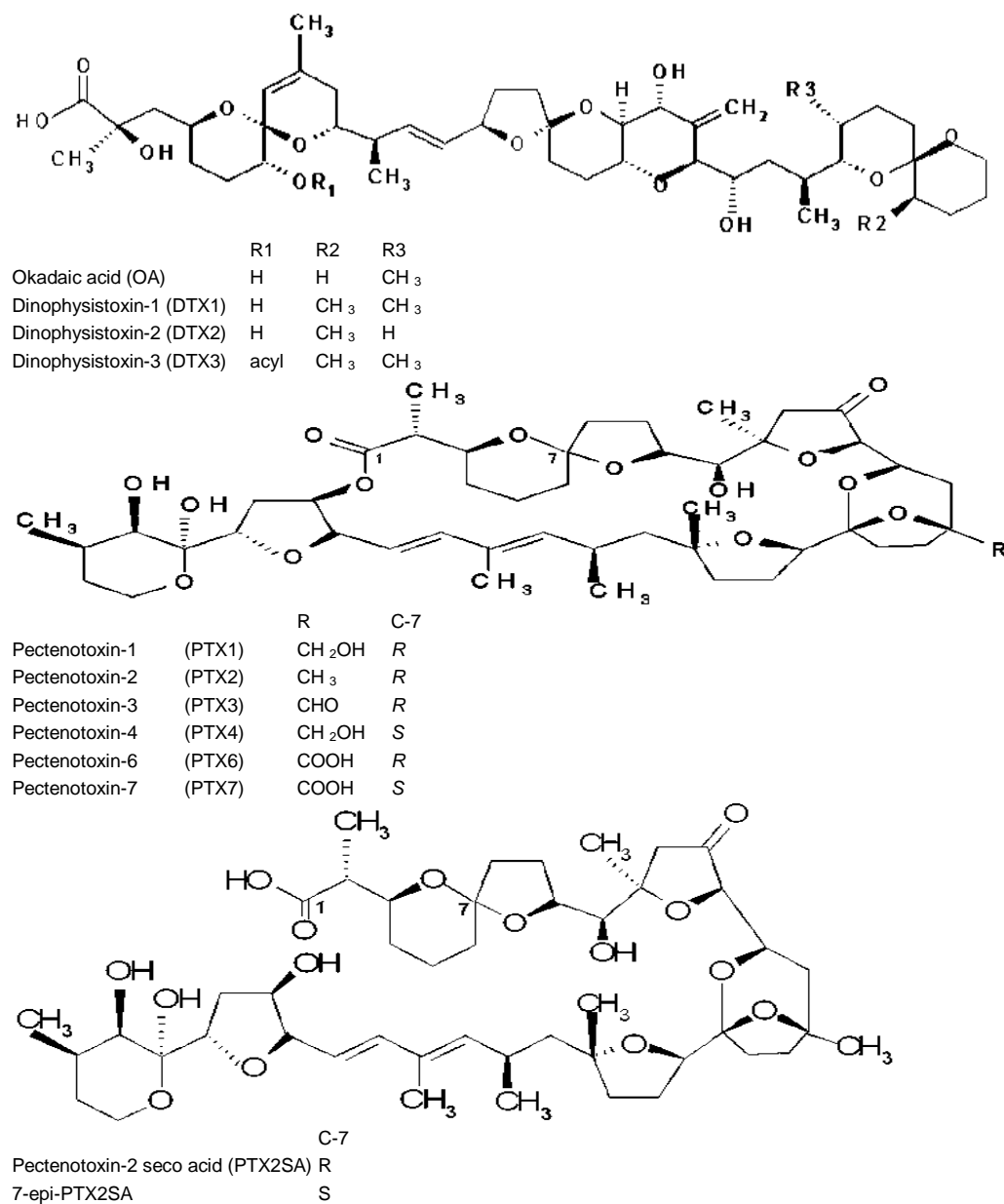
	R1	R2	R3
Okadaic acid (OA)	H	H	CH₃
Dinophysistoxin-1 (DTX1)	H	CH₃	CH₃
Dinophysistoxin-2 (DTX2)	H	CH₃	H
Dinophysistoxin-3 (DTX3)	acyl	CH₃	CH₃

		R	C-7
Pectenotoxin-1	(PTX1)	CH₂OH	R
Pectenotoxin-2	(PTX2)	CH₃	R
Pectenotoxin-3	(PTX3)	CHO	R
Pectenotoxin-4	(PTX4)	CH₂OH	S
Pectenotoxin-6	(PTX6)	COOH	R
Pectenotoxin-7	(PTX7)	COOH	S

	C-7
Pectenotoxin-2 seco acid (PTX2SA)	R
7-epi-PTX2SA	S

Figure 2. Chemical structures of okadaic acid, dinophysistoxins and pectenotoxins. Source: Yasumoto et al., 2001.

characterized by incapacitating diarrhea, nausea, vomiting, abdominal cramps and chills. Recovery occurs within three days, with or without medical treatment, (Climent and Lembeye, 1993; Climent et al., 2001; Clement, 1999; Cohen, 1974; Cosper et al., 1989; Currie et al., 2000).

Chemical properties

The DSP toxins are all heat-stable polyether and lipophilic compounds isolated from various species of shellfish and dinoflagellates (Draisci et al., 1996a) (Figures 2 and 3). Although diarrhea is the most characteristic symptom of intoxication, several other effects may be of relevance and some of the toxins in the DSP complex (PTXs and YTXs) do not yield diarrhea at all (Van Egmond et al., 1993). Re-evaluation of their toxicity will probably lead to these toxins being removed from their classification as DSP toxins (Quilliam, 1998a). The different chemical types of toxins associated with the DSP syndrome comprise:

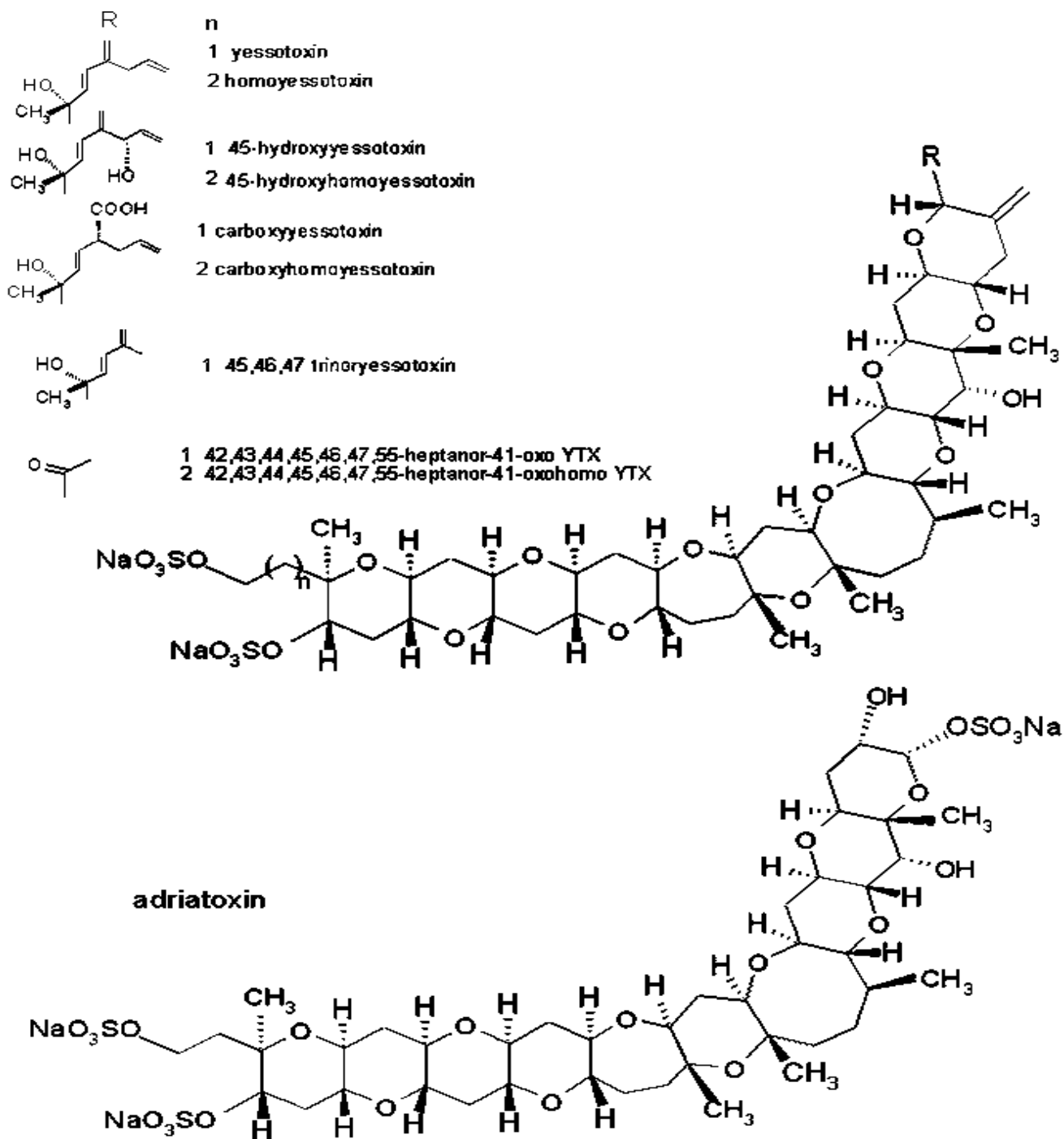a) The first group, acidic toxins, includes okadaic acid

Figure 3. Chemical structures of yessotoxins and adriatoxin. Source: Ciminiello et al., 1998; 2002 and Yasumoto et al., 2001.

(OA) and its derivatives named dinophysistoxins (DTXs). Okadaic acid and its derivatives (DTX1, DTX2 and DTX3) are lipophilic and accumulate in the fatty tissue of shellfish. These compounds are potent phosphates inhibitors and this property is linked to inflammation of the intestinal tract and diarrhea in humans (Van Apeldoorn et al., 1998; Hallegraeff et al., 1995). OA and DTX1 are also tumor promoters in animal test systems (Draisci et al., 1996a; Van Egmond et al., 1993). DTX1 was first detected in *Dinophysis fortii* in Japan; DTX2 was identified in shellfish in Ireland during a DSP episode (Van Egmond et al., 1993). DTX2 was isolated also from a marine phytoplankton biomass mainly consisting of *Dinophysis acuta* (James et al., 1999). A new isomer

of DTX2, named DTX2B, was isolated and identified in Irish mussel extracts (James et al., 1997). DTX3 originally described a group of DSP toxin derivatives in which saturated or unsaturated fatty acyl groups are attached to the 7-OH group of DTX1. More recently it has been shown that any of the parent toxins, OA, DTX1 and DTX2, can be acylated with a range of saturated and unsaturated fatty acids from C_{14} to C_{18} (Hallegraeff et al., 1995; Wright, 1995). In a report of an EU meeting, it was stated that chain length of the fatty acid can vary from C_{14} to C_{22} and that the number of unsaturation varying from 0 to 6. The most predominantly saturated fatty acid in DTX3 was palmitoyl acid (EU/SANCO, 2001). These acylated compounds also possess toxic activity. Since these compounds have only been detected in the digestive gland of contaminated shellfish, it has been suggested that they are probably metabolic products and not *de novo* products of toxin producing micro algae (Wright, 1995). Suzuki et al. (1998) demonstrated the transformation of DTX1 to 7-*O*-acyl-DTX1 (DTX3) in the scallop *Patinopecten yessoensis*. The ester bond in the acylated compounds can be hydrolyzed by heating in 0.5 M NaOH/90 percent methanol solution at 75°C for 40 min. The ester bond in DTX3 was also easily hydrolyzed by lipase and cholesterol esterase (EU/SANCO, 2001).

Two naturally occurring ester derivatives called diol esters were isolated from some *Prorocentrum* species. These diol esters did not inhibit phosphatase *in vitro*. However, it should be noted that these allylic diol esters may be somewhat labile and could be hydrolysed to yield the active parent DSP toxin (Hallegraeff et al., 1995). Draisci et al. (1998) reported the detection of another OA isomer and called it DTX2C. The structure of DTX2C is not yet elucidated. The compound was isolated from *Dinophysis acuta* collected in Irish waters.

b) The second group, neutral toxins, consists of polyether-lactones of the pectenotoxin group (PTXs). Ten (10) PTXs have been isolated until now and six out of these have been chemically identified; PTX1, -2, -3, -4, -6 and -7. Since PTX2 (PTX2,CH$_3$) is found in phytoplankton only (*Dinophysis fortii* in Japan and Europe) and never in shellfish, it is suggested that an oxidation occurs in the hepatopancreas of shellfish producing other PTXs (PTX1, CH$_2$OH; PTX3, CHO; PTX6, COOH) (Draisci et al., 1996a; Yasumoto et al., 2001; Van Apeldoorn et al., 1998). Sasaki et al. (1998) identified PTX4 and PTX7 as spiroketal isomers of PTX1 and PTX6, namely *epi*-PTX1 and *epi*-PTX6, respectively. Suzuki et al. (1998) demonstrated oxidation of PTX2 to PTX6 in scallops (*Patinopecten yessoensis*). Two new artifacts, PTX8 and PTX9, were also isolated but their structures are not yet elucidated. Daiguji et al. (1998) isolated two new pecteno toxins from the green shell mussel *Perna canalicus* from New Zealand and from *Dinophysis acuta* from Ireland and elucidated the structures as pectenotoxin-2-seco acid (PTX2SA) and 7-*epi*-pectenotoxin-2 seco acid (7-*epi*-PTX2SA), respectively.

c) The third group includes a sulphated compound called yessotoxin (YTX), a brevetoxin-type polyether, and its derivative 45-hydroxyyessotoxin (45-OH-YTX) (Draisci et al., 1996a; Van Egmond et al., 1993). Yessotoxin was first isolated from the digestive organs from scallops (*Patinopecten yessoensis*) in Japan (Ciminiello et al., 1999) and is believed to be produced by microalgae. The yessotoxins do not cause diarrhoea. Yessotoxin attacks the cardiac muscle in mice after intra peritoneal injection, while desulphated yessotoxin damages the liver (Van Egmond et al., 1993). In the digestive gland of Adriatic mussels (*M. galloprovincialis*) besides yessotoxin, two new analogues of yessotoxin, homoyessotoxin and 45-hydroxyhomoyessotoxin were identified by Ciminiello et al. (1997, 1999). Tubaro et al. (1998) also detected homoyessotoxin in *M. galloprovincialis* from the Adriatic Sea during a bloom of *Gonyaulax polyhedra* (*Lingulodinium polyedrum*). Satake et al. (1997) and Satake et al. (1999) isolated YTX and 45, 46, 47-trinoryessotoxin from cultured cells of the marine dinoflagellate *Protoceratium reticulatum*. The production of yessotoxins by *P. reticulatum* differed from strain to strain. Ciminiello et al. (1998) detected again a new analogue of YTX, adriatoxin (ATX), in the digestive glands of DSP infested Adriatic mussels collected in 1997 along the Italian coast (Emilia Romagna). In addition, four further analogues of yessotoxin, carboxyyessotoxin (COOH group on C_{44} of YTX instead of double bond), Carboxyhomoyessotoxin (COOH group on C_{44} of homoYTX instead of double bond) (Ciminiello et al., 2000a, b), 42,43,44,45,46,47,55-heptanor-41-oxo YTX and 42,43,44,45,46,47,55-heptanor-41-oxohomo YTX (Ciminiello et al., 2001, 2002) in Adriatic mussels (*Mytilus galloprovincialis*) were identified.

d) Unexplained human intoxication, with DSP symptoms, following the consumption of mussels from Killary, Ireland in 1995 was resolved by the isolation of a new toxin ($C_{47}H_{71}NO_{12}$), tentatively named Killary Toxin-3 or KT3 (Satake et al., 1998a).

NEUROTOXIC SHELLFISH POISONING (NSP)

Neurotoxic shellfish poisoning (NSP) is caused by toxins produced predominantly by *Gymnodinium* species like *Gymnodinium breve*, *Karenia brevis*. Several species of phytoplankton in New Zealand have been found to produce NSP toxins. These include *Gymnodinium c.f. breve*, *Gymnodinium c.f. mikimotoi* (which may include three separate species), *G. galatheanum* and a species of *Heterosigma* (Mackenzie et al., 1995a; Haywood, 1998). The identity of the causative agent in the 1993 NSP event in Northland is uncertain: both *Gymnodinium c.f. breve* and *Gymnodinium c.f. mikimotoi* were present in elevated numbers at the time (Chang, 1996; Mackenzie et al., 1995b).

Toxins produced: Brevetoxins

NSP produces an intoxication syndrome nearly identical to that of ciguatera. In this case, gastrointestinal and neurological symptoms predominate. In addition, formation of toxic aerosols by wave action can produce respiratory asthma-like symptoms. No death has been reported and the syndrome is less severe than ciguatera, but nevertheless debilitating. Unlike ciguatera, recovery is generally complete in a few days.

Monitoring programs (based on *Karenia brevis* cell counts) generally suffice for preventing human intoxication, except when officials are caught off-guard in previously unaffected areas (Passow, 1991; Perez et al., 2001; Rafuse et al., 2004; Schnorf et al., 2002; Tangen, 1977; Taylor et al., 1995).

Chemical properties

The NSP toxins, called brevetoxins, are tasteless, odorless, heat and acid stabile, lipid-soluble, cyclic polyether neurotoxins produced by the marine dinoflagellate such as *Gymnodinium breve* (or *Ptychodiscus breve*). The molecular structure of the brevetoxins consists of 10 to 11 transfused rings; their molecular weights are around 900. Ten brevetoxins have been isolated and identified from field blooms and *G. breve* cultures (Benson et al., 1999) (Figure 4). These brevetoxins show specific binding to site-5 of voltage-sensitive Na^+ channels leading to channel activation at normal resting potential. This property of the brevetoxins causes the toxic effects (Cembella et al., 1995). PbTx-2 is the major toxin isolated from *G. breve*.

Four brevetoxin analogues (Figures 5 and 6) were isolated from contaminated shellfish. The brevetoxin analogues were analysed in cockles (*Austrovenus stutchburyi*) (BTX-B1) (Ishida et al., 1995) and Green shell mussels (*Perna canaliculus*) (BTX-B2, BTX-B3 and BTX-B4) (Morohashi et al., 1995, 1999; Murata et al., 1998) and differed from brevetoxins isolated from dinoflagellate cultures. Apparently BTX-B1, BTX-B2, BTX-B3 and BTX-B4 are metabolites formed by the shellfish itself as they were not found in field blooms or *G. breve* cultures. The presence of BTX-B2, BTX-B3 and BTX-B4 in *Perna canaliculus* does suggest that metabolic pathways in this species are more complicated than those in cockles (*A. stutchburyi*). However, the major toxins in shellfish were left unelucidated because of the extreme difficulty in isolation (Morohashi et al., 1999).

In addition to brevetoxins, some phosphorus containing ichthyotoxic compounds resembling anti cholinesterases, have also been isolated from *G. breve* (Figure 7). One example is an acyclic phosphorus compound with an oximino group in addition to a thiophosphate moiety, namely O,O-dipropyl(*E*)-2-(1-methyl-2-oxopropylidene) phosphorohydrazidothioate-(*E*) oxime (Van Apeldoorn et al., 2001).

Paralytic shellfish poisoning (PSP)

Paralytic shellfish poisoning (PSP) toxins are present in some genera of dinoflagellates and one species of cynobacteri. Several species of the genus *Alexandrium* (formerly named *Gonyaulax* or *Protogonyaulax*) are identified as contaminators in shellfish. These are *Alexandrium tamarensis*, *Alexandrium minutum* (syn. *Alexandrium excavata*), *Alexandrium catenella*, Alexandrium fraterculus, *Alexandrium fundyense* and Alexandrium cohorticula. Other clearly distinct dinoflagellates have also been recognised as sources of the STXs.

These are *Pyrodinium bahamense* and *Gymnodinium catenatum* (Mons et al., 1998). The toxicity of the dinoflagellates is due to a mixture of STX derivatives of which the composition differs per producing species and/or per region of occurrence.

Toxins produced: Saxitoxins

PSP, like ASP, is a life threatening syndrome. Symptoms are purely neurological and their onset is rapid. Duration of effects is a few days in non-lethal cases. Symptoms include tingling, numbness, and burning of the perioral region, ataxia, giddiness, drowsiness, fever, rash, and staggering.

The most severe cases result in respiratory arrest within 24 h of consumption of the toxic shellfish. If the patient is not breathing or if a pulse is not detected, artificial respiration and CPR may be needed as first aid. There is no antidote, supportive therapy is the rule and survivors recover fully. PSP is prevented by large-scale proactive monitoring programs (assessing toxin levels in mussels, oysters, scallops, clams) and rapid closures to harvest of suspect or demonstrated toxic areas. Paralytic shellfish poisoning (PSP) has been reported to occur after eating puffer fish, filter feeding shellfish and molluscs.

If ingested by humans, PSP produces neurologic symptoms such as tingling and burning of the mouth and tongue, numbness, drowsiness and incoherent speech. These symptoms occur within 30 min to two hours after ingestion and in severe cases cause ataxia, muscle weakness, respiratory paralysis and death. The Toxic Exposure Surveillance System (TESS) of the American Association of Poison Control Centres has identified 10 illnesses of presumed puffer fish poisoning due to exposure from PSP after eating puffer fish from the area of Titusville, Florida, (Klöpper et al., 2003; Leikin and Paloucek, 1998; Lembeye et al., 1993; Lembeye, 1981; Luccas et al., 2005; MacKenzie et al., 1996; Mahoney et al., 1990).
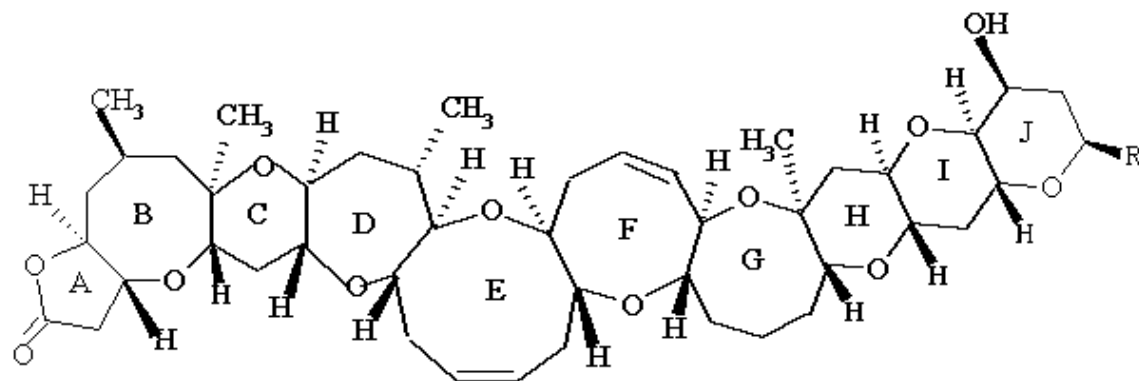
Type 1 (A) brevetoxins: PbTx-1,   R = CH₂C(=CH₂)CHO

PbTx-7,   R=CH₂C(=CH₂)CH₂OH

PbTx-10,   R=CH₂CH(CH₃)CH₂OH
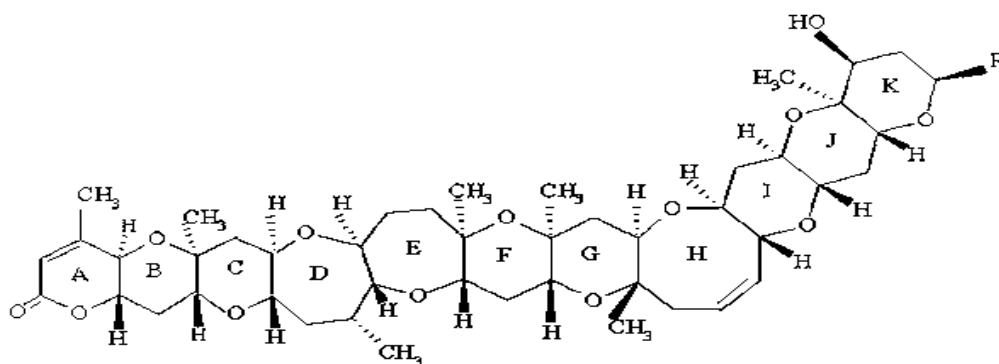
Type 2 (B) brevetoxins: PbTx-2   R = CH₂C(=CH₂)CHO

oxidized PbTx-2   R=CH₂C(=CH₂)COOH

PbTx-3   R=CH₂C(=CH₂)CH2OH

PbTx-8   R=CH₂COCH₂Cl

PbTx-9   R=CH₂CH(CH₃)CH2OH

PbTx-5   the K-ring acetate of PbTx-2

PbTx-6   the H-ring epoxide of PbTx-2

Figure 4. Chemical structures of type A and B brevetoxins (Hua et al., 1996).

Chemical properties

The PSP toxins form a group of closely related tetra hydropurine compounds that make up four subgroups: i) Carbamate (STX, neo STX and Gonyautoxins (GNTX1-4); ii) N-sulfo-carbamoyl (GNTX5-6, C1-4); iii) Decarbamoyl (dc-) (dcSTX, dcneoSTX, dcGNTX1-4); and iv) deoxydecarbamoyl (do-) (doSTX, doneoSTX and doGNTX1) components. At least 21 PSP toxins (Figure 8) mainly from marine dinoflagellates and shellfish that feed on toxic algae have been identified. Attempts to isolate PSP toxins began more than one hundred years
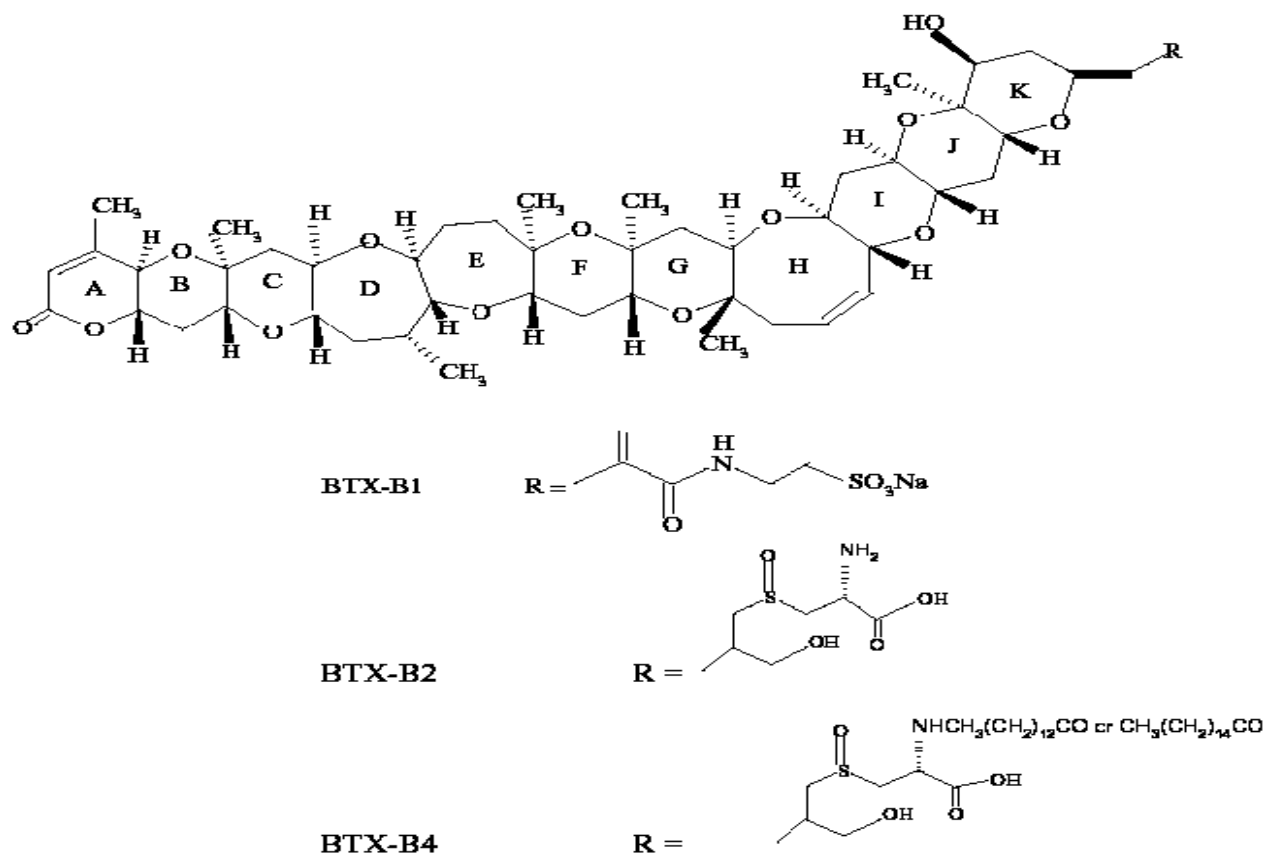
Figure 5. Chemical structures of brevetoxin analogues BTX-B1, -B2 and -B4 isolated from contaminated shellfish. Source: Yasumoto et al., 2001.

BTX-B3
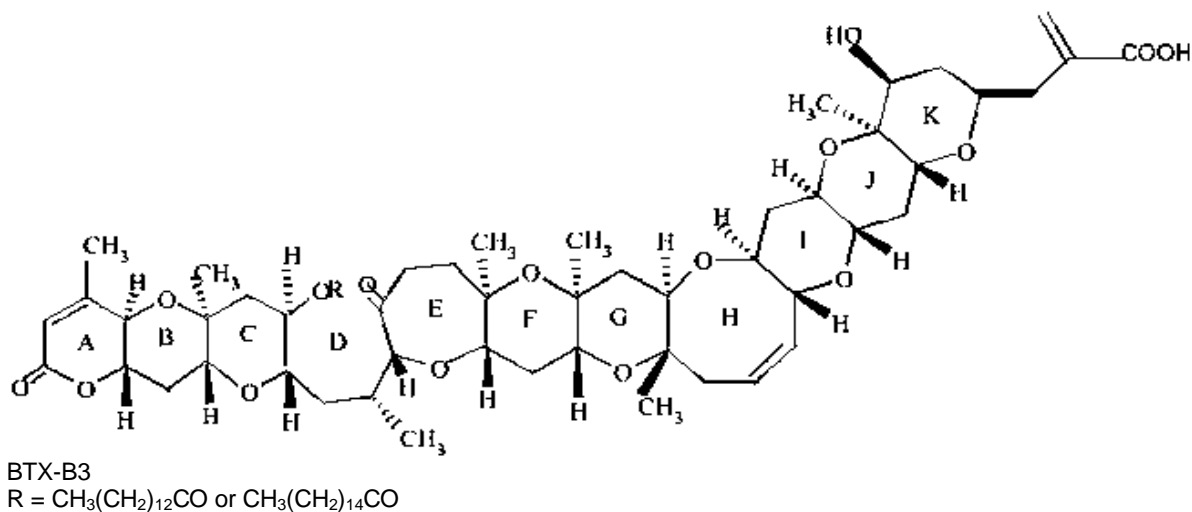R = CH$_3$(CH$_2$)$_{12}$CO or CH$_3$(CH$_2$)$_{14}$CO

Figure 6. Chemical structure of brevetoxin analogue BTX-B3 isolated from contaminated shellfish. Source: Yasumoto et al., 2001.

ago but their occurrence as mixtures of compounds with different ionizable functionalities complicated isolation procedures and early progress was slow. The development of ion-exchange chromatography, guided by mouse bioassays, eventually led to the isolation of a water-soluble basic toxin from the Alaska butter clam (*Saxidomus giganteus*). This compound was later given the trivial name saxitoxin (STX).
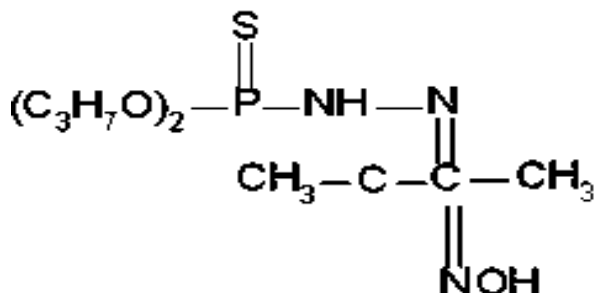
Figure 7. Phosphorus containing ichthyotoxic toxin isolated from *G. breve*. Source: Van Apeldoorn et al., 2001.

In 1975, the first crystalline derivative of STX was synthesized and the structure was studied (Bower et al., 1981). By means of X-ray crystallographic and nuclear magnetic resonance (NMR) spectroscopic studies the structure of STX was elucidated (Figure 8 for the chemical structures of STX and other PSP toxins). The dihydroxy or hydrated ketone group on the five rings is essential for its poisonous activity. Catalytic reduction of this group with hydrogen to a monohydroxy group eliminates the activity. Removal of the carbamoyl group side-chain on the six-membered ring, leaving a hydroxyl group in its place, produces a molecule with about 60% of the original toxic activity. The presence of this active hydroxyl group establishes a means for the preparation of various derivatives of STX (Mons et al., 1998). The PSP toxins are heat stable at acidic pH (with the exception of the N-sulfo-carbamoyl components) but unstable and easily oxidized under alkaline conditions (Mons et al., 1998).

Amnesic shellfish poisoning (ASP)

Amnesic shellfish poisoning (ASP) is caused by Domoic acid (Wright et al., 1989). Species within the genus *Pseudo nitzschia* produce Domoic acid. This toxin is unusual in being produced by diatoms rather than dinoflagellates and some causative organisms like *Pseudo nitzschia australis*, *Pseudonitzschia pungens*.

Toxins produced: Domoic acid

ASP can be a life-threatening syndrome. It is charac- terized by both gastrointestinal and neurological dis- orders. Gastroenteritis usually develops within 24 h of the consumption of toxic shellfish; symptoms include nausea, vomiting, abdominal cramps and diarrhea. In severe cases, neurological symptoms also appear, usually within 48 h of toxic shellfish consumption. These symptoms include dizziness, headache, seizures, disorientation, short-term memory loss, respiratory difficulty, and coma.

In 1987, four victims died after consuming toxic mussels from Prince Edward Island, Canada. Since that time, Canadian authorities have monitored both the water column for the presence of the causative diatom, and shellfish for the presence of the toxin, domoic acid. Shellfish beds are closed to harvesting when the domoic acid concentration reaches 20 µg/g shellfish meat. Fish and crab viscera can also contain domoic acid, so the risk to human consumers and animals in the marine food chain is more significant than previously believed (Rafuse et al., 2004; Schnorf et al., 2002).

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Chemical properties

Discrimination between some species of *Pseudo- nitzschia* is virtually impossible under a light microscope because of morphological similarity between species- some species differ in details that can only be detected under an electron microscope. However, whole cell DNA probes have been developed to distinguish between species (Rhodes et al., 1998), and these are utilised by industry in risk management when deciding whether to implement voluntary closures to harvesting, pending the results of shellfish toxicity testing.

Amnesic shellfish poisoning (ASP) is caused by domoic acid (DA) (Figure 9), a naturally occurring compound belonging to the kainoid class of compounds that have been isolated from a variety of marine sources including macro- and microalgae (Wright and Quilliam, 1995). DA is a crystalline water-soluble acidic amino acid. It can be purified by a variety of chromatographic methods and contains a strong chromophore that facilitates detection by UV spectroscopy.

DA was originally discovered as a product of a red macroalgae *Chondria armata* and was later isolated from several other red macroalgae. However, these seaweeds were not the source of DA in the first reported ASP incident on Prince Edward Island in Canada in 1987. The source of DA in this outbreak of ASP was found to be the diatom Pseudo- *nitzschia* (formerly *Nitzschia*) pungens forma multi series. DA is a potent neurotoxin and the kainoid class of compounds to which DA belongs, is a class of excitatory neurotransmitters that bind to specific receptor proteins in neuronal cells causing continual
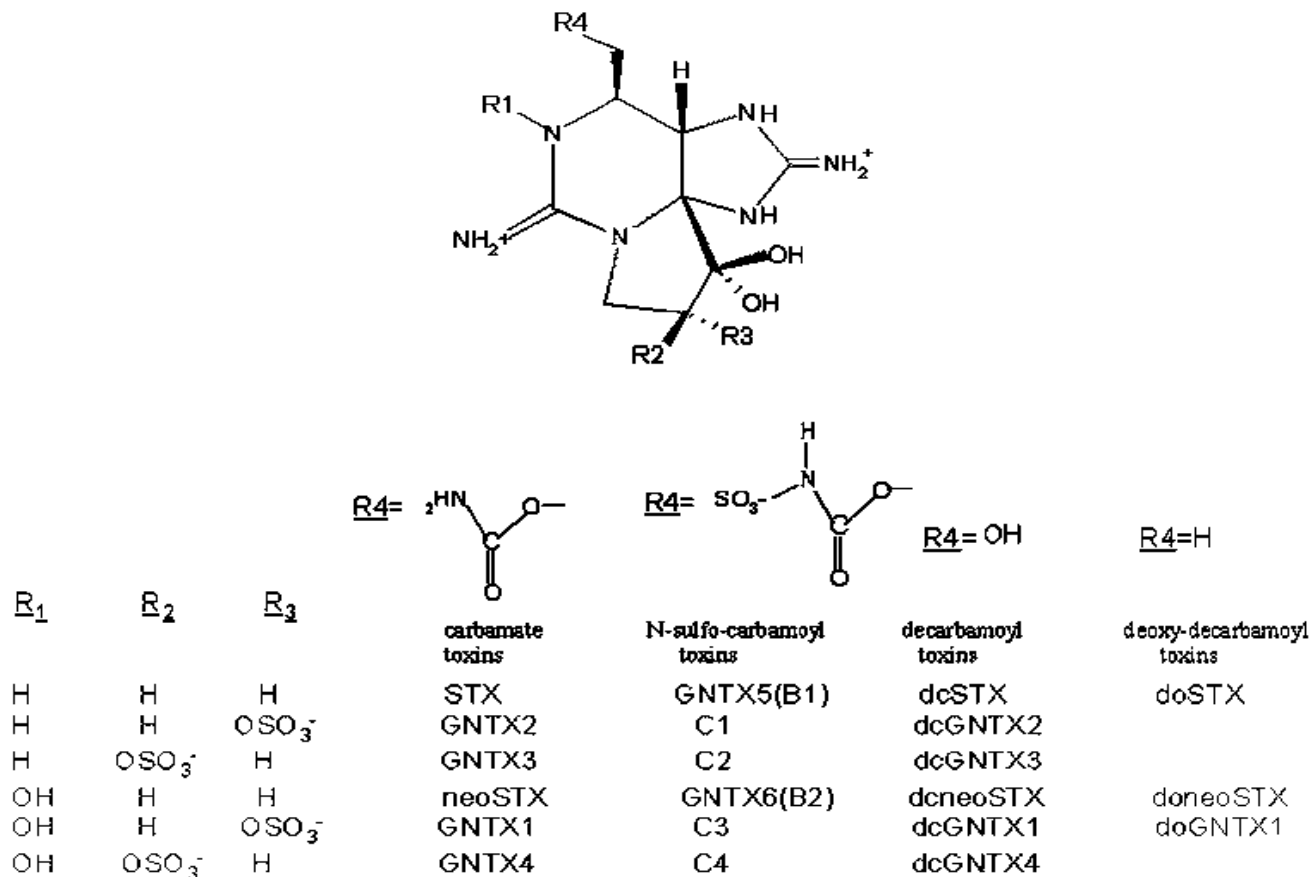
R_1	R_2	R_3	carbamate toxins	N-sulfo-carbamoyl toxins	decarbamoyl toxins	deoxy-decarbamoyl toxins
H	H	H	STX	GNTX5(B1)	dcSTX	doSTX
H	H	OSO_3^-	GNTX2	C1	dcGNTX2	
H	OSO_3^-	H	GNTX3	C2	dcGNTX3	
OH	H	H	neoSTX	GNTX6(B2)	dcneoSTX	doneoSTX
OH	H	OSO_3^-	GNTX1	C3	dcGNTX1	doGNTX1
OH	OSO_3^-	H	GNTX4	C4	dcGNTX4	

Figure 8. Chemical structures of PSP toxins. Source: Mons et al., 1998; Quilliam et al., 2001.

depolarization of the neuronal cell until cell rupture occurs (Wright, 1995).

Investigation of the kainoids present in *Chondria armata* resulted in the discovery, in minor amounts, of the geometrical isomers isodomoic acid A, B and C (Figure 9) as well as domoilactones. None of these isomers, found in seaweed, were detected in extracts of plankton or shellfish tissue. However, three other geometrical isomers (isodomoic acids D, E and F) and the C5' diastereomer (Figure 8) were isolated from both plankton cells and shellfish tissue (Wright and Quilliam, 1995; Ravn, 1995). The geometrical isomers can be prepared in the laboratory by brief exposure of dilute solutions of DA to UV light, and are therefore not considered to be *de novo* products of the plankton. Pharmacological studies indicate that these photoisomers bind less strongly to the kainate receptor proteins than DA itself suggesting that they are not as toxic as the parent amino acid. Formation of the C5' diastereomer is accelerated with warming. This C5' diastereomer shows almost the same binding efficacy to the kainate receptor as DA itself (Wright and Quilliam, 1995). Zaman et al. (1997b) reported the isolation of two new isomers of DA from the red alga *Chondria armata*, i.e. isodomoic acid G and H (Figure 9).

DETECTION OF MARINE TOXIC

In all cases, the marine HAB toxins that cause the human poisoning syndromes consist of families or groups of structurally related compounds, with individual derivatives exhibiting potencies that can significantly differ from other congeners (Van Dolah, 2001). During food web transfer, HAB toxins can also be metabolized or bio-transformed into structurally different compounds. The broad chemical and structural diversity of algal toxins and their derivatives and metabolites, coupled with differences in their potency account for many of the challenges associated with their detection in ocean observatory programs. Traditionally, biotoxin monitoring programs have relied on measurements of toxins in shellfish samples collected weekly or bi-weekly from key locations in areas affected by HABs (Shumway et al. 1988).

Toxin measurement methods can be grouped into three main types: chemical, *in vitro*, and *in vivo* assays (Hallegraeff et al., 2003). The latter (bioassays) have had a long history in HAB toxin detection, but are obviously not amenable to automation and high-throughput analysis in ocean observatories, so the only options in that context are measurements of toxin in seawater using either
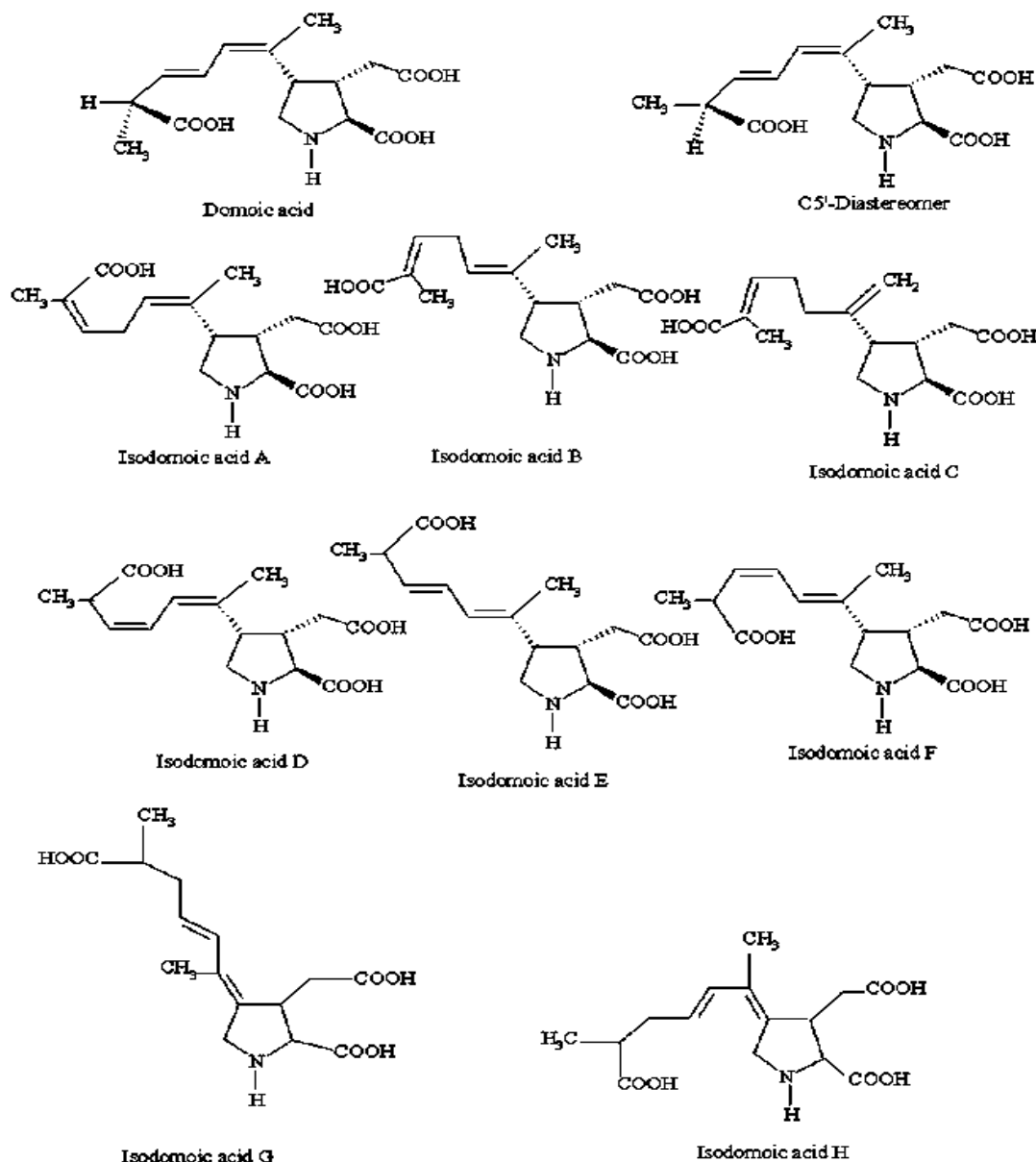
Figure 9. Chemical structures of domoic acid and its isomers. Source: Wright and Quilliam, 1995, Zaman et al. (1997b).

chemical analyses or *in vitro* assays. This immediately introduces some concerns, as considerable work will be needed to relate measurements of toxins dissolved in seawater, or in particulate form in that water, to the risk to human consumers of shellfish or fish.

Chemical methods for toxin analysis include high performance liquid chromatography (HPLC), and mass spectrometry coupled to liquid chromatographic separation (Quilliam 1996). Of these two alternatives, only mass spectrometry shows the potential for use in ocean observatories, and the challenges remain significant due to the diversity, size, and solubility of the toxins, as well as the matrices in which they occur (for example, parti-

culate versus dissolved). Another constraint is the need to perform spectrometry in a vacuum and underwater, which poses significant engineering challenges. Progress has been good, however. For example, a small, modular mass spectrometer has been developed and mounted in an Autonomous Underwater Vehicles (AUV) (Wenner et al., 2004). That system consists of an *in situ* membrane-introduction linear-quadrupole mass spectrometer capable of detecting dissolved gases and volatile organic compounds at sub parts-per-billion concentrations. This instrument is still under development and has not been configured for HAB toxins, but future designs may permit the analysis of HAB toxins that occur dissolved in seawater

(for example, brevetoxins, domoic acid, okadaic acid).

Analysis of toxins in particulate form will require a different approach, such as laser desorption mass spectrometry (LDMS), which is widely employed in analytical laboratories due to its simplicity of operation and rapid analysis times. One benefit of LDMS is that many different types of materials can be vaporized and ionized by a tightly focused laser beam (Cotter 1997). This can avoid sample purification or preparative techniques, which is critical to deployment of such technologies in a moored or mobile configuration in an Ocean Observing System (OOS), as it will greatly reduce sampling and handling requirements, and thus power drain, space needs, and reagent needs as well. LDMS has been used for the detection of bacterial spores, vegetative cells, viruses, and toxins in aerosol environments (Fenselau and Demirev, 2001), and efforts are underway to apply this method to HAB cells and dissolved toxins in seawater.

Another important and rapidly developing group of HAB toxin detection methods comprises the *in vitro* assays. One subgroup the functional assays, relies on detection of a toxin's biochemical activity while the other structural assays depends on recognition of chemical structure at the molecular level (Cembella et al., 2003; Van Dolah and Ramsdell, 2001). A variety of functional assays have been developed for the detection of HAB toxins, including cyto toxicity assays (Manger et al., 1995), enzyme inhibition assays (Della Loggia et al., 1999), and receptor binding assays (Van Dolah et al., 1994). Nevertheless, retention of the biological activity of a cell line or a receptor preparation outside the laboratory remains a significant, and thus far, insurmountable obstacle to *in situ* use of these assays (Sellner et al., 2003).

In contrast, structural assays show considerable promise for automated deployment in an observatory system. These assays rely on the structural or conformational interaction of a toxin with a recognition factor such as an antibody. Antibody-based assays have been developed for a variety of HAB toxins and many of these tests are now commercially available (Laycock et al., 2001; Cembella et al., 2003). One novel immunoassay utilizes surface plasmon resonance (SPR) in a portable system developed for rapid field quantification of toxin levels in both shellfish and seawater (Stevens et al., 2007).

The SPR assay had a limit of detection of 3 ppb domoic acid and a quantifiable range from 4 to 60 ppb. Comparison of analyses with standard HPLC protocols gave an excellent correlation. This same technology should also function for detection of domoic acid (and other algal toxins for which antibodies are available) in concentrated algal extracts or high dissolved levels in seawater. With refinement of the extraction protocols and generation of higher affinity monoclonal antibodies, detection of much lower levels of toxin should be possible, leading to eventual application of automated SPR

biosensors on moorings. Another novel and potentially useful approach for *in situ* observations is a competitive immunoassay using screen printed electrodes (SPEs; Kreuzer et al., 2002; Micheli et al., 2004). Excellent sensitivity and accuracy has been achieved with HAB toxins such as okadaic acid, brevetoxin and domoic acid and for all toxins investigated, results compared favourably with other toxin analysis techniques. The advantages of speed of analysis, simplicity of design, *in situ* measurement capability, stability (storage up to four weeks prior to use), and disposability make screen printed electrodes (SPE) immune sensors good candidates for observatory instrumentation. Adaptation of this and other immunoassay technologies to robotic systems and deployment in remote locations is thus possible, but will require further development effort.

CONCLUSION

Only a small number of HAB species can be detected using optical measurements, either *in situ* or remotely from space, and therefore instruments that can detect the vast majority of HAB species need to have capabilities for sample collection, concentration, and manipulation. The chemistries and procedures for cell identification and enumeration using molecular probe assays of various types are well established.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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